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# A COMPLEMENT-FIXATION TECHNIC FOR THE DIAGNOSIS OF WEIL'S DISEASE*

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The clinical laboratory is frequently asked to assist in the differtial diagnosis of jaundice. The available tests at best determine whether or not the jaundice is hepatocellular, obstructive, or hemolytic in type. All too frequently the laboratory can offer no information concerning etiologic agents. In Weil's Disease, such an opportunity for etiological diagnosis presents itself. The disease, while not common, should be considered in all types of jaundice not clearly of obstructive or hemolytic types. Three general methods are available for the detection of the presence of this organism: 1. Demonstration of the organism in direct dark-field examination of blood and urine; 2. Detection of the organism by animal inocculation; 3. The detection of antibodies to the Leptospira by the agglutination and lysis test.

While the first two of these methods offer the most specific

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means of diagnosis, the actual demonstration and identification of these organisms is fraught with some difficulty and requires perhaps more training and equipment than may be available. Although of slightly less absolute diagnostic significance, the demonstration of antibodies to the disease is more suitable to the routine laboratory than the more tedious method of animal inoculation and perhaps is more precise than the identification of L. hemorrhagiæ by the occasional examiner. For the detection of antibodies, the agglutination-lysis test has been used extensively in experimental work. However, its use in routine clinical examinations suffers the same limitations as the actual demonstration of the organism, namely, considerable skill and experience are required to perform the test.

The complement-fixation test has largely been neglected in diagnostic and experimental work, although Noguchi[1] and others have shown that the complement-fixation test will also detect Leptospira antibodies. Some difficulty has been encountered with the reaction, but Besseman and Nelis[2] state that the complement-fixation reaction appears to be a fairly reliable test, provided a pure culture of the spirochete is used as antigen, rather than a liver extract.

It occurred to us that if a suitable antigen could be obtained that the complement-fixation test would be more practicable for routine use in clinical laboratories. It would eliminate the necessity of cultivating the organisms, since the antigen could be obtained from a central source, and the technic is similar to that now used in the performance of Wassermann tests. Even though our studies are limited, they have been very encouraging. We submit this report with the hope that it will stimulate others to use the test in routine and experimental work so that its real value as a diagnostic procedure may be ascertained.

*Antigen*

The first antigens were prepared from broth cultures of L. icterohemorrhagiæ and L. canicola supplied by the Department of Bacteriology of the Woman's Medical College of Pennsylvania. These cultures were grown in Schuffner's modification of Verwoort's medium. Nursing bottles containing 5 oz. of medium were inoculated with 1 cc. of Leptospira culture and allowed to grow at room temperature for a week. Each culture was examined by dark field for active Leptospira and tested for absence of other organ-

isms. The cultures were heated at 100° C. in the Arnold sterilizer for two hours, then either centrifuged or filtered through a Seitz bacteriological filter. The supernatant fluid was removed and merthiolate added to final concentration of 1-5000. We have also used broth cultures of L. hemorrhagiaæ and L. canicola supplied by the Pathological Department of the Veterinary School, University of Pennsylvania.

The broth antigens prepared in this manner showed no anti-complementary activity in 0.5 c.c. amounts and all were antigenic in doses ranging from 0.1 to 0.025 c.c. Antigenic titrations are conducted by testing a positive serum with 0.2, 0.1, 0.05, and 0.025 amounts of the antigen.

There is evidence as shown in Case 1 that the complement-fixation test is species specific. It is therefore advisable to use both L. icterohemorrhagiæ and L. canicola antigens, as reactions are dependent upon which of these organisms is the cause of the infection.

*Simplified Complement-Fixation Technic for Leptospirosis*

1. The preparation of sheep cells, hemolysin, saline and complement is the same as recommended for the Boerner-Lukens Wassermann test.[3]

2. The Leptospira antigen is substituted for the B-J-L antigen.

3. The hemolytic system is adjusted as described in the Boerner-Lukens test.

4. The Leptospira antigen is added separately, therefore, the antigen-complement mixture is omitted.

*Qualitative Test Using Human Serum*

1. For each serum to be tested, two tubes are used (Kahn or Kolmer). To each tube add 0.1 c.c. of serum. Place in water bath at 58° C. for ten minutes, or at 56° C. for one-half hour to inactivate.

2. Include three controls—antigen, hemolytic and corpuscle.

3. To both tubes, add 0.5 c.c. of 1:30 complement.

4. To tube one, add 0.2 c.c. of Leptospira antigen.

5. Mix by shaking and place in water bath at 37° C. for one hour.

6. To both tubes, add 0.5 c.c. of 0.75 per cent sensitized cells. (Cells should sensitize at least fifteen minutes before using.)

7. Mix by shaking and place in water bath at 37° C. for one hour.

8. Read reactions as follows:

| | |
|---|---|
| Plus four | = Positive |
| Plus one, two and three | = Weakly positive or doubtful |
| Complete hemolysis | = Negative |

*Quantitative Test Using Human Serum*

1. For each serum to be tested, three or more tubes are used.

2. To all tubes except the last, add 0.2 c.c. of saline.

3. Add 0.2 c.c. serum to tube one and 0.1 c.c. to the last tube.

4. Mix tube one and transfer 0.2 c.c. to tube two; mix tube two and transfer 0.2 c.c. to tube three; and continue transferring until the next to the last tube is reached; then discard 0.2 c.c. from this tube.

5. Continue in same manner as described under qualitative test.

TABLE I

COMPLEMENT FIXATION TEST (QUALITATIVE)

| Tube | Serum | Comp. 1:30 | Antigen | | Sensitized Corpuscles 0.75 per cent | |
|---|---|---|---|---|---|---|
| | c.c. | c.c. | c.c. | | c.c. | |
| 1 | 0.1 | 0.5 | 0.2 | Incubate | 0.5 | Incubate |
| 2 | 0.1 | 0.5 | — | 1 hour | 0.5 | 1 hour |
| Ant. Cont. | 0.1* | 0.5 | 0.2 | at | 0.5 | at |
| Comp. Cont. | — | 0.5 | — | 37°C | 0.5 | 37°C |
| Corp. Cont. | 0.5** | | | | 0.5 | |

*—Negative serum
**—Saline

*Case 1*

On June 21, 1940, we received a blood specimen from a case of suspected Weil's disease at the Philadelphia General Hospital. The patient presented typhoid-like symptoms with jaundice and had been sick about ten days. The complement fixation test was positive (plus 4) with antigens of L. icterohemorrhagiæ and L. canicola. On June 25, the agglutination-lysis test with L. icterohemor-

rhagiæ was positive (plus 4) in a dilution of 1 to 30,000 and with L. canicola 1:300.

On April 18, 1941, post-recovery tests were performed. The complement fixation test was still positive to L. icterohemorrhagiæ, but entirely negative to L. canicalo; and the agglutination-lysis test showed a titer of 1 to 300, with L. icterohemorrhagiæ, and 1:30 with L. canicola.

TABLE II

QUANTITATIVE COMPLEMENT FIXATION TESTS

Case Number 1*

Ten Months After Recovery

| | Serum | | | | |
|---|---|---|---|---|---|
| Antigen 0.2 c.c. | 0.1 | 0.05 | 0.025 | 0.0125 | Control |
| L. icterohemorrhagiæ #1 | +4 | +4 | +4 | — | — |
| L. icterohemorrhagiæ #2 | +4 | +4 | 1 | — | — |
| L. icterohemorrhagiæ #3 | +4 | +4 | ± | — | — |
| L. icterohemorrhagiæ #4 | +4 | +4 | ± | — | — |
| L. canicola #1 | — | — | — | — | — |
| L. canicola #2 | — | — | — | — | — |

*Agglutination-lysis titer:

L. hemorrhagiæ =1:300
L. canicola =1:30
Wassermann test=negative

*Case 2*

Through the courtesy of Dr. George P. Perakos of New Britain, Connecticut, we received blood serum from a patient who had recovered from Weil's disease. From the clinical data, together with the blood studies made at the National Institute of Health, there was little doubt as to the correctness of the diagnosis, even though the organism was not recovered by animal inoculation. The patient's disease began on August 1, and he was seriously ill for over three weeks. He subsequently recovered, and has remained in good health to the present time.

On August 23, 1940, he agglutinated L. hemorrhagiæ in a serum titer of 1:150,000.

On January 20, 1941, we tested his serum and it gave a positive (plus 4) reaction in the complement fixation test. At the same

time, the agglutination titer had dropped to 1:10,000.

On April 5, 1941, we received another specimen of his serum. This gave a positive (plus 3) reaction in the complement fixation test and an agglutination titer of 1:3000.

The agglutination tests on August 23 and January 20 were performed at the National Institute of Health and the last one on April 5 by Dr. E. L. Stubbs.

### *Specificity Tests*

A group of 109 medical students were tested for Leptospirosis by the technic described, and all gave entirely negative reactions.

During the last year, cases with varying degrees of jaundice including both hepato-cellular and obstructive types, were tested with entirely negative results.

On October 25, 1940, we tested nine sera received from the Pennsylvania Department of Health. These were from a district near Williamsport, Pennsylvania, where some type of infectious jaundice has been endemic. The histories of these cases are as follows:

Case 1—Age 40, jaundice over July 4, 1940.

Case 2—Age 20, attack one year ago—nausea, vomiting, and jaundice.

Case 3—Age 12. This boy had had his infection two weeks following his playmate's infection, about one year ago. Headache, nausea and jaundice.

Case 4—Age 17, jaundice, nausea, and vomiting in September, 1940. Has not felt well since.

Case 5—Age 13. Took ill September 4 and was sick two weeks with jaundice.

Case 6—Age 48, jaundice, nausea, and vomiting for two weeks.

Case 7—Age ?, jaundice, nausea, and vomiting during February, 1940.

Case 8—Age 16. Had jaundice during July, 1940, with nausea and vomiting.

Case 9—Age 17, jaundice, nausea and vomiting during May, 1940.

It was thought that if these were cases of Weil's Disease some

would still show the presence of antibodies. Blood specimens were collected, and one part of the blood was sent to the National Institute of Health in Washington for the agglutination test, and the other part was sent to us for the complement-fixation test. The result was that they were all negative to the complement-fixation test, and also the agglutination test performed by the National Institute of Health, which strongly indicated that this endemic disease is not Weil's disease.

*Discussion*

In the beginning, we used a primary incubation of eighteen hours at 8° C. With this procedure, many of the Wassermann positive sera reacted to the Leptospira antigen. It was hoped that the overnight incubation could be used so it would only be a matter of changing the antigen in those laboratories conducting the Wassermann test. With one hour incubation at 37° C, the cross reactions with Wassermann positive sera were eliminated. In a series of tests in which twenty-five sera, giving strongly positive Wassermann reaction were included, all gave negative reactions to the Leptospira antigen. The occurrence of this cross reaction with Wassermann positive sera after eighteen hours incubation at 8° C, seemed to indicate that Leptospira and Spirocheta pallida have something in common in their antigenic structure. Costa and Troisieri[4] have found that the Wassermann reaction may become positive in Weil's Disease, a fact in accord with the previous statement. These findings suggest the advisability of performing a complement fixation test for syphilis on suspected Weil's Disease sera.

In Case 1, when the antibody titer was high, we obtained cross reactions against both L. canicola and L. hemorrhagiæ antigens. Unfortunately, we did not perform a quantitative test at the time. In the post-recovery tests, when the antibody titer had diminished, the reaction was positive only with the L. hemorrhagiæ antigen. At this time, the titer (agglutination-lysis test) was 1-300 and 1-30 respectively for the L. hemorrhagiæ and L. canicola, as indicated in Table 2. This suggests that at the height of the clinical disease, the antibodies to L. hemorrhagiæ were most increased and perhaps at that time, a quantitative complement fixation test might have detected this species difference. Similarly, the results in Table No.

2 indicate that the complement fixation test is positive when the agglutination-lysis antibody titer is positive between 1-30 and 1-300.

*Conclusion*

1. Broth cultures of Leptospira hemorrhagiæ and Leptospira canicola were found suitable as antigens for use in the complement-fixation test.

2. The complement-fixation test revealed Leptospira antibodies when the agglutination and lysis test was as low as 1:300.

3. The complement-fixation test is suggested for trial as a routine clinical laboratory procedure for the diagnosis of Weil's Disease.

We wish to express our appreciation to Drs. Clara Raven, Ruth A. Miller, and Mary E. McKee Porter, Woman's Medical College of Pennsylvania, for supplying us with cultures of Leptospira; and to Drs. E. L. Stubbs and David Coffin, Veterinary School, University of Pennsylvania, who, in addition to supplying cultures, performed the agglutination and lysis tests.

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# A STUDY OF HEMATOPOIESIS IN A GROUP OF FEMALE STUDENTS AGES FIFTEEN TO TWENTY-THREE YEARS THROUGH A STUDY OF GASTRIC SECRETIONS AND CORRELATED BLOOD COUNTS*

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That a report such as this is may not be disappointing, it may be well to remind the reader that the data accumulated has been made on young ladies who were engaged in the strenuous duties of school life in the college and college preparatory schools,—obviously such a life must indicate sound health, and hence any positive findings must be very scant in the report herewith submitted. In other words, the source of material has been taken from what may well be considered the best soil in the world.

It has been said by a great doctor that no children of any other generation have had the start in life of the present. Health statistics note that this is the age of almost negligible contagious diseases; the ebb of rheumatic fevers is low; the greatest start in balanced diets seems now prevalent. On the other hand, offsets to such aids to good health are dietary fads, fear of obesity, subtle smoking which tends to cut down the appetite, and lastly, the lack of adequate rest. All of these offsets are perhaps more often seen among individuals who because of close contact do of necessity imitate each other.

This paper is a report on a survey made on female students between the ages of fifteen and twenty-three years inclusively, who volunteered to participate in the better health program of the school

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year 1941. The project consisted in trying to establish a correlation between the acidity of the gastro-intestinal tract and the condition prevalent in the blood stream. Each volunteer was given, upon an empty stomach, a test meal consisting of six arrowroot cookies and 500 cc. of water. In the first twenty-five subjects, this was expressed in thirty minutes time, but for reasons to be discussed, the digestion time was prolonged to equal or exceed forty minutes. Simultaneously with the expression of the stomach, hemoglobin determinations and red and white blood cell counts were made.

All this work was done with very little exception between 8:00 and 9:00 A.M. Of the 100 volunteers, 45 of the apparently abnormal individuals were called back for a repeat gastric expression in a month's time. Of these, 5 were recalled for the third time. In every instance the blood was checked a second time after a month's interval and, where discrepancies occurred, rechecking continued until results appeared satisfactory. Two hundred forty-three complete blood determinations, consisting of hemoglobin, red blood cell count, and white blood cell count; 150 gastric expressions; and 98 chemical analyses for iron were made.

The equipment for the blood determination was standardized before any of it was used. For hemoglobin determinations, a Haden-Hausser hemoglobinometer and a Sahli-Leitz instrument were used as checks against each other. The gastric expression was made by means of a nasal catheter and a 50 cc. syringe. The iron determinations were made on blood collected under fasting conditions or nearly so, using stainless steel needles, 10 cc. glass syringe, and sodium citrate to the extent of 0.2 per cent. Ten cc. of blood were collected, 2 cc. being immediately transferred to a 35 cc. test tube, the remainder being centrifuged and the plasma removed immediately. The total gastric expression was allowed to stand by necessity about an hour so that with the exception of a few specimens, the aliquots for titration were obtained by pipetting off the supernatant fluid which had formed a layer above the remains of the cookies. Töpfer's method for determination of free hydrochloric acid and combined acids was followed. Three aliquots were titrated on every specimen, and the readings for free hydro-

chloric acid taken at the canary-yellow end point.[1]

The readings for the three titrations were averaged and these averages were used in the discussion that follows.

### *Gastric Secretion Data*

A study of this kind is not easy just because of the difficult access to gastric secretions, especially in women and girls. It is quite certain that the gastric juice varies little in the same individual from day to day. On this assumption, since the gastric expressions were made under identical conditions on the same individuals but at interims of two to four weeks, they were considered representative. It was found, however, that before the laboratory data on gastric analyses had any meaning, the results of certain vitiating factors had to be eliminated. Those factors in the light of a series of repeated expressions on the same individual were, in all probability:

1. Inadequate time for digestion of test meal;

2. Incomplete evacuation of stomach contents following digestion or duodenal regurgitation subsequent to stomach evacuation;

3. Mucous interference. Data is submitted to illustrate the vitiating influence or lack of it due to one or the other of these factors.

*1. Time:*

When all stomachs are studied under identical conditions, i.e., the same quality and quantity of food, the same time interval of digestion, true values are not obtained of the degree of acidity because of differences in speed of elaboration. Recognition of this

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[1] Over 600 qualitative determinations were made to ascertain the correctness of canary-yellow as an end point for free hydrochloric acid. In nearly every instance it took a drop or more of 0.1 normal sodium hydroxide after the appearance of the canary yellow color to completely neutralize the free hydrochloric acid.

normal variation in elaboration is given by Rehfuss.[2]

Fourteen cases were studied with the consideration of the effect of increased time for digestion of the test meal on the acid production. The distribution of these cases is as follows:

TABLE I

GASTRIC SECRETIONS VERSUS TIME

| Minutes of Digestion | Initial Degrees Total Acidity With No Increases Upon Additional Digestion of Ten or More Minutes | | | | | |
|---|---|---|---|---|---|---|
| 30-35 min. | 10-20° | 21-30° | 31-40° | 41-50° | 51-60° | 61-70° |
| | 1 | 7 | | | 1 | 1 |
| | With Increase in Acidity Upon Ten Minutes Additional Digestion | | | | | |
| | | 10° inc. 1 | | | | |
| | | 20° inc. 1 | | | | |
| | With Increase in Acidity Upon Twenty Minutes Additional Digestion | | | | | |
| | | 10° inc. 1 | | | | |
| | | 20° inc. 1 | | | | |

Ten of the cases showed no increase in acidity upon additional time for digestion and distributed themselves in the extreme areas of total acidity—viz., in the hypochlorhydric and hyperchlorhydric areas. This may mean that such individuals will give as representative an acid finding in thirty as in forty to forty-five minutes of

[2] Preferably both in health and in disease when referring to stomachs secreting less than 25 degrees free hydrochloric acid following forty-five to sixty minutes of digestion on a test meal, the term hypochlorhydric is used, and when secreting over 50 degrees, hyperchlorhydric. The absence of free hydrochloric acid is referred to as achlorhydria. Isosecretory is being applied to stomachs elaborating between 25 and 50 degrees free hydrochloric acid. With these meanings hyperchlorhydric, hypochlorhydric, isosecretory, and achlorhydric are used in this treatise.

digestion; the data of the ten subjects with a digestion period of thirty minutes, showing no alteration in acidity upon additional digestion, were not eliminated from further consideration in the study.

If the degree of acidity is graphed against time, the height of secretion is not reached in the slowly elaborating stomachs as quickly as in the rapidly elaborating ones. While both hyposecretory and hypersecretory stomachs begin to give representative readings in thirty minutes, isosecretory need nearly twice the time.

The other four cases in the table showed an increase in acidity upon additional digestion time, the increase being due to free hydrochloric acid only. Obviously thirty minutes is not sufficient time for such slow elaborating stomachs to produce representative secretions and these four cases were eliminated from further consideration in this study.

*Time:* The following cases were typical of the ten in which additional time made no alteration:

| Subject | Min. of Digestion | Vol. in cc. | Total Acidity |
|---|---|---|---|
| M.J. | 30 | 233 | 17 |
| | 45 | 105 | 19 |
| P.K. | 30 | 100 | 70 |
| | 40 | 90 | 63 |

While the cases summarized below are typical cases of those needing extension of digestion time to yield representative acidity values:

| | | | |
|---|---|---|---|
| B.B. | 30 | 205 | 27 |
| | 55 | 175 | 36.5 |
| E.J. | 30 | 210 | 26 |
| | 45 | 70 | 46 |

The data below is being submitted on digestive periods in excess of forty minutes. Typical readings to indicate nature of findings on remainder of subjects are as follows:

| Minutes of digestion | Total acidity in degrees |
|---|---|
| 50 | 14 |
| 50 | 40 |
| 50 | 45 |
| 50 | 50 |
| 55 | 36.5 |
| 55 | 41 |
| 65 | 56 |
| 75 | 58 |
| 42 | 36 |
| 40 | 36 |
| 45 | 36 |
| 45 | 29.5 |
| 45 | 47 |
| 40 | 40 |

In averaging several findings for representative intervals of time, the following is obtained:

| Time Interval | Average Degrees Total Acidity |
|---|---|
| 40 min. | 38° |
| 45 min. | 37° |
| 50 min. | 40° |

From these figures it seems obvious that a fraction of the subjects needed somewhere between forty and fifty minutes.

Since no absolute correlation between time and acidity seems to exist, with the exception of the ten cases with thirty minutes of digestion, all cases kept in the study represent periods of digestion of forty or forty-five minutes. It now seems safer never to attempt a gastric expression on digestion periods less than forty to forty-five minutes.

II. *Volume:*

The volume of the contents of a digesting stomach at any given time is dependent on the stimulus. Food is the normal stimulus, but histamine hypodermics and alcohol stomach gavages are effective. The volume is also dependent on the size of the meal, the time interval of digestion, and the tonicity of the pylorus which tonicity is physiologically dependent on the pH of the stomach contents.

Although water leaves the stomach rather rapidly by excursion[1] through the pylorus, the question arises whether there had been complete evacuation of the test meal water in thirty minutes. The acceptable volume in a normal stomach after forty-five to sixty minutes upon 250-400 cc. fluid intake is about 100 cc. The volume obtained in this study after digestion upon 500 cc. fluid intake averaged from 195 cc. for lower age span to 183 cc. for higher age span. It may be a volume characteristic for females between fifteen and twenty-three years, or it may be due to the volume of moisture-absorbed cookies, cookies being almost completely retained because of the recognized retarded digestion; or, it may be due to some retention of water from the test meal. If the last factor is the cause, then all the acidities reported are lower than normal.

Typical laboratory data were at hand in which volume discrepancies appeared. Two possible explanations are offered for these discrepancies.

1. Incomplete evacuation of the test meal water. This was hardly probable, for water leaves the stomach fairly rapidly.

2. Regurgitation from the duodenum, and in this study, 7 per cent gave indications of what appeared to be regurgitation—bile tinged fluid of disproportionately low acidity. Any such findings were eliminated from the laboratory data discussed in this report.

*III. Mucus:*

Another vitiating factor in the interpretation of the laboratory data was introduced by the presence of an abnormal amount of mucus. In these specimens, ropes of mucus, not the ordinary filmy type that settles out, but tenacious masses were expressed with difficulty. One subject complained of pain in the region of the stomach while expression was in progress. The picture suggests more than ordinary salivation provoked by nervousness, and the excess mucus may have interfered with titration. At least 4 out of the 100 volunteers manifested an excess amount of ropey mucus. These specimens were likewise eliminated from the report of this study.

When all indicated eliminations had been made from the laboratory data, the remaining figures for gastric expressions were mathe-

matically treated and compared for health evaluation with available normals. Normal values submitted by a group of workers[1] from the Mayo Clinic of Rochester, Minnesota, were used in spite of certain differences in the technical production of the statistics in the tables. Among these differences were: 1. Their test meal, which consisted of 8 arrowroot cookies and 400 cc. of water given on an empty stomach and expressed one hour after ingestion; 2. In the Mayo Clinic study, *apparent* achlorhydria was defined as no free hydrochloric acid in the first hour, and *true* achlorhydria as no free hydrochloric acid obtained either the first hour or on subsequent expressions made every fifteen minutes without re-inserting the tube; or expressions made subsequent to histamine injection. For this study: 1. The test meal was not identical; 2. Apparent achlorhydria had the same meaning; 3. True achlorhydria meant no free hydrochloric acid in any of a series of repeated expressions upon re-insertion of tube. Most of the Rochester cases of apparent achlorhydria proved to be true achlorhydria. Sixty-six per cent of our apparent achlorhydrias proved to be true achlorhydrias.

The Mayo Clinic experiment has pointed out that there is a straight line correlation between the incidence of achlorhydria and age, and that females show 3 per cent higher values than males. Free gastric acidity appeared to increase rapidly from the age of childhood up to the age of 20, when adult values were reached. (Combined acids, according to Heath and Patek,[3] should show no significant variations at any age, the average being about 17 degrees, and those with achlorhydria should show only slightly less combined acids than those without it.)

The Rochester normals were compiled from some 3,000 records selected from many more on apparently normal people who passed through the Mayo Clinic, on whom gastric analyses had been made.

With respect to the section from the Rochester[1] normals now being used for comparison, the Rochester experimenters say: "We found (referring to their search through Mayo Clinic records for normal gastric analyses) so few persons with ages less than twenty years that a satisfactory statistical record of their measurements could not be made. We searched the literature for gastric analyses

made on presumably normal . . . youths . . . We used the data thus obtained to fill the gap in our material." In spite of this admission that for the types of individuals now under study there is little on record, their report is being accepted as normal for comparative purposes.

TABLE II
GASTRIC SECRETION DATA COMPARED

| | Age 15-19 years inc. | | Age 20-23 years inc. | |
|---|---|---|---|---|
| | Mayo Clinic | Test Subjects | Mayo Clinic | Test Subjects |
| Incidence of no free HCl | 2% | 0% | 4% | 4% |
| Mean free HCl | 50.7° | 21.78° | 48.2° | 23.74° |
| Standard of deviation | 16.2° | 8.3° | 16.2° | 8.6° |
| Number of cases studied | 42 | 45 (one 14-year old) | 90 | 55 |

Obviously the student body under study falls far below these normals. Before correct conclusions can be drawn, three considerations must be made: 1. The normals may be at fault; 2. Actually the students tested may be collectively hypochlorhydric; or 3. The fluid content and time of digestion of test meal may have altered values.

*Hematological Data*

Blood for hemoglobin and counts is more easy of access than gastric juice and hence records of hematological findings do not contain the element of chance to vitiate their face values. The data of this study were so treated as to yield the tabulation graphed on Table III and Chart I which follow.

In the building of normals for hemoglobin, cognizance has long been given to the fact that the quantity varies normally with sex, age, occupation, and geograpical location.

From the extensive work done in determining hemoglobin by oxygen capacity and photometric methods, Haden, Osgood, Wintrobe and Miller, Appleton, Williamson, and Haden and Neff, by

working on different ages in the two sexes, and in different geographical locations, by averaging their separate values have reported their combined findings.[2] Some of the desired details are not available.

Moore, Minnich, and Welch[5] have given hematological data on sixteen young ladies, freshmen in college; and Jenkins and Don[2] submit data on English women. For comparative purposes, the findings of this study are tabulated against the data of these various experimenters.

TABLE III

HEMATOLOGICAL DATA COMPARED

| | Haden, Osgood et al | Jenkins and Don | Test Subjects | |
|---|---|---|---|---|
| Age in years | 15-25 | "adult" | 14-19 inc. | 20-23 inc. |
| Hb. range (Gm.) | 14.5-15.2 | 10.78-17.22 | 10.8-14.7 | 10.4-14.8 |
| Mean Hb. (Gm.) | ? | 14.0 | 12.725 | 12.57 |
| Stand. Hb. deviation | ? | ±1.16 gm. | ±0.7 gm. | ±0.88 gm. |
| Normal range (Gm.) | ? | 12.838-15.16 | 12.02-13.42 | 11.69-13.45 |

| | Moore et al. | Test Subjects | |
|---|---|---|---|
| Age | "College freshmen" | 14-23 yr. inc. | |
| | | Unselected | Selected[a] |
| Hb. average (Gm.) | 13.1 | 12.44 | 12.60 |
| R.B.C. average (in million per cu. m.) | 4.27 | 4.21 | 4.25 |
| C.I. average | | 1.017 | 1.014 |

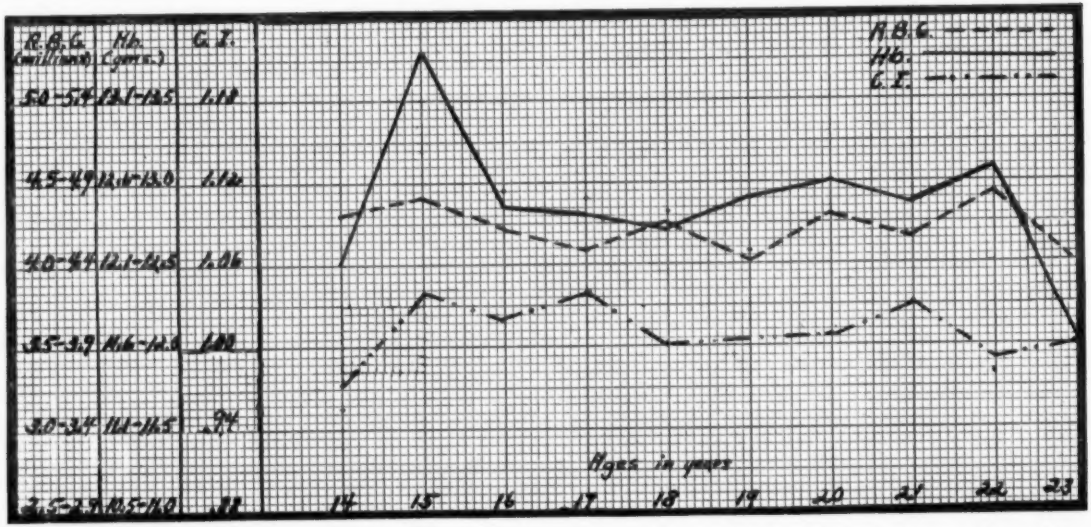
The hematological data from the student-subjects of this project place the greater number of students in the narrow range of 12 and 13 gm. of hemoglobin, which is in the lower normal range in both England and America.

From the nature of the color index, the red blood cell count would automatically fall below normal had more "normals" been

[a] All of these subjects who subjectively or objectively were suffering from blood pathology or gastro-intestinal disorders, or whose kin in the first degree were thus suffering, were eliminated and those not so eliminated were considered "selected."

CHART I

HEMATOLOGICAL DATA




| No. of Determinations | 3 | 21 | 32 | 25 | 15 | 14 | 38 | 55 | 15 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|

available.

Color indices reported in this study have been calculated on a hemoglobin coefficient of 14.5 gms. (i.e., 14.5 gm./100 cc. blood on the basis of red cell count of 5,000,000/cu.m.). The normal color index is 1.0, but the figures from 0.85-1.15 are not abnormal.[2] In pathological cases of iron deficiencies, a range of 0.3-0.8 with an average of 0.56 has been reported. In this study the lowest C.I. was 0.80, the highest, 1.24, with the general distribution as graphed in Chart I.

Normal white cell counts are reported as ranging somewhere between 5,000 and 10,000. [2, 3] White cell count may have no value in the study of hematopoiesis when the emphasis is on hemoglobin production. Especially in acute iron deficiency diseases there is no characteristic white blood cell count, or if there is any alteration, it is usually in the direction of a decreased count, and in chronic iron deficiency, it may be reduced as low as 2,500.[3] The average w.b.c. count in this study was 6,600, the general distribution was as follows: The lowest single count was 4,400, and the three highest single counts exceeded 9,000.

White Cell Count Distribution
Per cu. m.
(220 Determinations)

| 4,000-4,900 | 5,000-5,900 | 6,000-6,900 | 7,000-7,900 | 8,000-8,900 | 9,000-9,900 | 10,000 |
|---|---|---|---|---|---|---|
| 4% | 21% | 37% | 22% | 9% | 6% | 1% |

*Hypochromic Anemia*

The laity's most frequent diagnosis of a faulty hematopoiesis is anemia. This is made because the individual looks pale, acts tired, and his general state of nutrition is low. Young women of the age span covered by this report are traditionally anemic—a kind of anemia uniquely their own, an anemia in which the formation of hemoglobin lags behind the formation of red blood cells. As first pointed out by Osler in 1885, the cells are pale or achromic and usually small. Red blood cells of such blood usually have a color index (ratio of their color to that normally found) of 0.6 or less.[3] An anemia of such a description is referred to as a hypochromic anemia—an iron deficiency anemia because the condition improves with iron therapy.

Hypochromic anemia, in fact any anemia is recognized by clinical changes; some or all of which may be seen in any one case, involving the tongue, eye, nervous system, gastro-intestinal tract, as well as the color and condition of the skin and the blood.[2] Especially in hypochromic forms of anemia, the tendency has been to stress gastro-intestinal disorders as a change causing the faulty hematopoiesis. As the purpose of this paper is primarily to correlate in the process of hematopoiesis the acid secretory function of the gastro-intestinal tract with the facts revealed by blood counts, only these features and iron metabolism will be emphasized.

Hypochromic anemias are classified as several, but they can well be enumerated as types of the same disease—iron deficiency—occurring at different ages, or in different physiological, physical, and pathological (disease processes) conditions.[3] Hence, when iron requirements reach their highest, and other conditions are identical, hypochromic anemias are seen most commonly. On the basis of weight, iron requirements reach their highest in both sexes in the first two years of life, at the time of puberty, during adolescence, and in the event of frank blood loss when the emergency occurs. However, starting with puberty, the female's iron needs begin to exceed the male's and when both have reached twenty years of age, the female is requiring at least four times as much iron as the male, and this ratio persists until the menopause.

The iron requirement at two years goes into the increasing volume of blood and mass of hemoglobin that accompanies growth; that at the time of puberty and continuing through adolescence into the steady growth of body; the excess requirement in the female into the menses, and during any frank blood loss or in chronic insidious bleeding, into the recovery of lost iron, because blood loss means iron loss, blood being five times richer in iron than any other tissue.

A healthy individual in good nutrition can lose one-half his blood volume and reestablish his former hemoglobin level, providing his hygiene is excellent otherwise, by supplies from without by means of an adequate diet; and from within, by means of the iron stores of the body. It requires about three weeks after blood loss for

iron to reach the lowest level it maintained in health.[3] On the other hand, an individual whose iron stores are depleted, even with the proper diet may be anemic for months and years. About 25 mg. of iron are needed to increase the hemoglobin 1 per cent.[3] If less than 10 mg. of iron are available for hemoglobin synthesis per day, it may take a month to build the level 10 per cent.

Regarding the normal loss by menses, Ohlson and Daum[4] working on "normal" women disclose the following for four menstrual periods, giving the loss in hemorrhage in terms of mg. of iron as 18.16, 25.68, 32.0 and 41.0 mg. respectively. Barer and Fowler[4] reporting on 98 cases stated the average blood loss per month was 50.55 cc. representing 22.2 mg. of iron and 6 gm. of hemoglobin, which in terms of standard in Duluth, viz. 14.5 gm./100 cc. as normal, the hemoglobin drop is about 41 per cent per month if the iron level were not sustained from adequate sources. If women were to lose only 20 mg./mo. of iron through menses, they have to absorb 1 mg./da. of iron to make up for this need alone.[4] Women's intake of iron is less than that of men. The average iron intake according to Widdowson and McCance is:

| Sex | Mg./da. | Mg. of which is available |
|---|---|---|
| Male | 16.8 | 10.9 |
| Female | 11.4 | 7.9 |

Thus it becomes evident that anemic tendencies in women are greater because the predispositions are greater: blood loss, less intake, and of this intake lesser quantity available than in a male.

The iron deficiency anemia of puberty was the first type of iron deficiency anemia to be recognized as a clinical entity. In the severe condition formerly prevalent, it was called "chlorosis" and its mild form, it is relatively common today.

Of the severe form of chlorosis, Clark W. Heath says: "I do not believe that it (chlorosis) was due to living in garrets, tight lacing, or reading unhealthy literature, although I believe that poor general hygiene and insufficient diets were undoubtedly responsible for many cases. Today girls and women are largely free from the protective barriers of a Victorian age, exercise and health are empha-

sized, and information regarding the importance of diet to health has been widely disseminated, and these factors have much to do with the better health of young women.[3]"

In reviewing the literature, it is seen that the attempt to find the cause of hypochromic anemia has been made on females of or near the middle age. From the facts concerning them, it seems safe to deduce similar facts relative to girls from fifteen to twenty-three years inclusive, except that whatever causes are revealed they are less marked, for in youth, when one body constituent is disturbed, another functions in its place. With advancement in age there is less and less of this accommodation.

In the older persons, when obvious causes for hypochromic anemia such as malnutrition, hemorrhage, infection, nephritis, cancer of bone marrow, or a variety of leukemias are excluded, the tendency is to stress achlorhydria or hypochlorhydria together with, or independent of the diet. This practice is so prevalent that hypochromic anemias with achlorhydria or hypochlorhydria are being set aside as a different and distinct class in which achlorhydria presumably plays a role, secondary or primary, in the cause; a secondary role only when in the mind of some, mal-absorption is considered primary.

To confirm their contentions that this is true, they offer several facts, viz.:

1. It is a very common occurrence to find patients who demonstrate no other cause for their anemia to be unable to secrete hydrochloric acid in the stomach.

2. In anemias associated with pregnancy, dietary deficiencies predominate mal-absorption from the gastro-intestinal tract being associated with decreased gastric acidity.

3. In hypochromic anemia of middle-aged women, hypochlorhydria or achlorhydria is seen in most cases.

4. Even when these middle-aged women with achlorhydria manifest no symptoms of anemia, they still behave differently than their equals upon some provocative cause, such as chronic or acute

blood loss. They can no longer sustain their former apparently normal hemoglobin level, and anemia develops. While this is attributed to low levels in iron reserves, the question arises, why should there be low iron reserve in these specific cases?[3] On this point, Heath[3] says that more work is needed to show the function or non-function, availability or non-availability of stored reserve iron.

5. Another fact presents itself, that of heredity. Whatever is the cause or purpose in nature that certain individuals have little or no free hydrochloric acid in their stomach and yet live normally is a question that may remain unanswered, yet the fact that anemic tendencies are encountered in other female members of the same family argues for an hereditary factor.[3] It is possible for hypochromic anemia to eventually shade off into pernicious anemia in the same individual, or one female may have pernicious anemia in conjunction with another female of the same family having hypochromic anemia.

6. After gastrectomy and other gastric operations, a hypochromic anemia develops which responds to iron therapy.

7. It is proven by experiment that in treating iron deficiency anemia, the reticulocyte response following iron therapy is usually less satisfactory in a patient with hypochromic anemia demonstrating achlorhydria than in one who normally secretes hydrochloric acid in the stomach.

8. Hypochromic anemia with achlorhydria requires larger doses of iron for hemoglobin regeneration than do cases of hypochromic anemia without achlorhydria.

On the other hand, facts may be presented that disprove achlorhydria as the primary or even secondary cause of hypochromic anemia:

1. Achlorhydria and hypochlorhydria may be found normally as a very benign symptom, the incidence increasing with age.

2. Achlorhydria and anacidity seem to be a manifestation of some remote cause that brings on such diseases as pellagra, cancer, nephritis, arthritis, some forms of hypersensitivity such as hay

fever, the achlorhydria disappearing with the recovery of the disease. Thus, anemia is not the only disease characteristically associated with acid deficit.

3. Absence of free hydrochloric acid in the stomach may be associated with anemias of one or more types.

4. It appears that anemia itself often predisposes to anacidity.[3]

5. Achlorhydria and intestinal disorders are more common in the population which subsists on milk and cereal diet.

Hypochromic anemia, it has already been pointed out, has for one of its causes blood loss as hemorrhage or through "spilling" itself into newly acquired growth tissue. Additional causes have been deduced from other facts and a composite picture of the whole phenomenon seems to be:

Blood loss in presence of iron stores—no anemia.

Blood loss in absence of iron stores—hypochromic anemia.

No blood loss in absence of iron stores—hypochromic anemia.

The hypochromic anemia developed may be of two natures determined by hemoglobin regeneration on an adequate diet. The hemoglobin regeneration may be:

1. Gradual, coming back to normal after months or longer, or

2. Not at all. If not at all, achlorhydria is usually present. As a proof Mettier[3] fed for sixty days, three cases on a diet calculated to yield 15-20 mg. or iron/da. The hemoglobin response during that period was as follows: Case No. 1, drop from 60 per cent to 55 per cent; Case No. 2, rise from 42 per cent to 45 per cent; Case No. 3, rise from 55 per cent to 60 per cent. This marks no significant response on hemoglobin regeneration on diet alone. Subsequent to the expiration of the sixty days, the diet was supplemented with iron citrate by mouth and in thirty additional days the hemoglobin went from 55 per cent to 73 per cent; from 42 per cent to 80 per cent; and from 60 per cent to 92 per cent respectively.

In the uncomplicated hypochromic anemia, inorganic iron by mouth in adequate doses, 15 grains of ferrous salt or larger doses of ferrous ammonium citrate by mouth produced 1 to 3 per cent rise in hemoglobin per day. This was proven by the three cases of Mettier's just discussed.

The reason for the different response to adequate diet unsuppplemented and supplemented gives a second cause for hypochromic anemia. Thus far only blood loss has been stressed. Now in order of emphasis these two may be presented as:

Cause No. I = blood loss (Minot) (Heath)
Cause No. II = Mal-absorption, either because of
a) No iron to absorb
b) Iron to absorb, but not in absorbable state

That there is iron to absorb is the merit of the diet and
That there is no iron to absorb may or may not be the fault of the diet.

In the sixty cases reported by Heath and in the cases reported by Mettier, the matter of diet was given consideration. In any cases with marked hypochromasia (color indices averaging 0.57) there was definitely little or no iron to absorb. In nearly every instance the diets were low in animal protein; meat, milk, and eggs being absent or not indulged in oftener than three times a week; or they were lacking in fresh non-processed foods such as colored vegetables and fresh fruits; or they were made up with substitutions of much bread, potatoes, and highly refined products, or sometimes the diets were the tea and toast type. Sometimes the calories were adequate for most of the women were of normal weight or overweight. (In this study we are able to report that out of the 100 students 3 of the young ladies who had symptoms of hypochromic anemia were also overweight.) Sometimes the calories were too low; this was pointed out by Heath as a usual failure of girls in their teens.

When by merit of the diet there is iron to absorb but it is not in an absorbable condition, it is the fault of the gastro-intestinal tract. In the embryological sense the gastro-intestinal tract is still outside the body and unless this important organ is properly preparing the iron in the food administered for absorption and subsequent assimilation, it cannot be certain that an adequate diet will produce adequate blood formation.[3]

Inadequate absorption may be due to:

1. Lack of acid in the stomach.[3] The role which the free hydrochloric acid plays in rendering the iron of food absorbable is twofold: (a) It dissolves the iron out of the food if not already dissolved, and (b) Keeps it in the ferrous state in the absence of

oxidants. Since the consensus of opinion is that it is in this form that it is absorbed and that the greater part is absorbed from the duodenum, in the duodenum the acid further prohibits precipitation until the absorption has been accomplished.[5] In a stomach without free hydrochloric acid, the pH is above 5, a hydrogen ion concentration that favors the formation of insoluble and undissociated iron compounds; obviously such forms can never be absorbed.[4]

2. Anatomical changes in the stomach. These are being visualized better since the rediscovery of the gastroscope.

3. Anatomical changes of the wall of the small bowel.

4. Abnormal diminution of the motility of the small bowel.

5. Excess mucus. The mucus may be present more extensively in the stomachs of patients suffering from hypochromic anemia than in patients suffering from pernicious anemia when it is scant.

In order of time, various approaches have been made to study the degree of iron absorption from the gastro-intestinal tract.[5] The first attempt was to check excretion of iron by way of feces and urine against oral ingestion—the inference being that the difference represented the amount absorbed. The fallacy in this method is that no account is made of the iron that may be absorbed from the intestine and re-excreted into the intestine.

The second attempt was to check the amount of iron that disappeared from an isolated segment of intestine, arguing that the difference between the injected and the recovered iron represented the absorption. The possibility of mucosal absorption was overlooked.

The third approach was by checking hemoglobin increase and accrediting the rise to iron absorption. The error in this method lies in the fact that not always does all the iron absorbed go to make hemoglobin.

With respect to relative rate of absorption, Moore is convinced that all depends on the ease with which iron can be reduced to the ferrous state in the intestinal tract, for water soluble ferrous salts were absorbed in anemic cases proportionately as well in the absence of acid as in the presence of normal acidity; and, that ferric salts responded correspondingly if reducing substances, as Vitamin C were fed simultaneously with the iron. Otherwise, they were

not absorbed. This emphasizes the need of the ferrous and not of the need of the hydrogen iron in the intestinal tract.

If the picture of why there is or is not hypochromic anemia is to be carried to completion the assimilation of iron after absorption must be detailed into it. Once iron is absorbed, it circulates in the plasma in the ferric state, usually as an undissociated complex with phosphorus-containing compounds as nucleic acid, phosphatides, and phospho-proteins. It is not ultra-filtrable and until it is dissociated and reduced to the ferrous state it cannot be utilized. Perhaps Vitamin C or the proteins of the blood stream accomplish this reduction.[4] Once the reduction has been accomplished the liver does much in the preliminary construction of the hemoglobin, suitable for the finishing touches by the bone marrow.[4] That the liver can normally accomplish this, there must be a continuous cycle of secretion and absorption of bile from the intestinal tract.

Whipple has shown in his experimentation on dogs that a number of substances in the presence of iron have had an added effect on hemoglobin production and he enumerates certain foodstuffs: liver extracts, bile pigments, chlorophyll, and chlorophyll derivatives.[3] While this may be true Moore asserts that these same bile pigments and chlorophyll derivatives do not help in the absorption from the intestine.

The thought that hypochromic anemia is caused by excretion of iron is entertained by no one. Iron is a "one-way" substance—it can enter the body but it cannot be excreted in appreciable quantities except by hemorrhage. Most of the iron administered orally leaves the body by way of the feces, but excretion of metabolic iron in the feces, urine and bile is very small—almost negligible.

The intestinal mucosa may under certain circumstances and within certain limits absorb or reject iron according to the body's needs, hence controlled absorption rather than excretion regulates the amount of iron in the body.

Conclusively it may be said with Minot that hypochromic anemia is the cumulative effect of:

1. Blood loss
2. Deficient diet
3. Gastro-intestinal disorders, and
4. Faulty iron metabolism.

Heath appends such other possible factors as the effects of vitamins, hormones and an otherwise healthy metabolism. Without an adequate diet, proper iron absorption and generally good metabolism, anemia cannot be avoided, or if developed cured through iron therapy.

*Distribution of Iron in the Body*

A normal human being is said to possess 4.3 gm. of iron. This is distributed as follows:

1. Hemoglobin (heme or hematin) the circulating or functioning iron constituting about 2.7 gm. or approximately 90 per cent of the total iron in the body. At one time a determination of hemoglobin was thought to be quite an accurate determination of functioning iron.

2. Parenchyma or tissue iron which is unavailable for blood formation—about 0.3 to 0.6 gm. which corresponds in amount to iron of body tissue following depletion of the readily available iron stores.

3. Depot iron which is a latent form of iron stored in the liver, spleen and marrow for emergency purposes, about 1.3 gm. but in rare occasions, a store (hematochromatosis) as large as 50 gm. in a single organ[3] has been reported.

4. Barkan[5] has shown another variety that is chemically unstable in dilute mineral acids. Some of this variety is a ferrous iron complex which he calls fraction E and another ferric iron complex which he calls fraction $E^1$, both of which reside in the red blood corpuscle and collectively have been called by other experimenters non-hemoglobin corpuscular iron (N. H. C. Fe.). When fraction $E^1$ disrupts it releases a form of iron which becomes the major portion of plasma iron. Abderhalden as early as 1898 showed there was more iron in the blood of various animals than could be attributed to hemoglobin alone.

A. Plasma iron:[5] Plasma iron or serum iron as a whole is sometimes called iron in transit. It is really a reflection of the iron absorbed from the gastro-intestinal tract; and, is in equilibrium with iron that may be used for hemoglobin synthesis, iron on its way to being excreted, iron on its way to become in part of the iron storage depot, or iron from red blood corpuscles on its way

to become bile pigments. Hitherto, plasma iron has been regarded as insignificant, but in the course of preliminary study of anemias, Jenkins and Thomson have shown that plasma iron varies significantly and can be influenced by various kinds of treatment. This makes an excellent tool for registering the fact that there is absorption and the degree, but it fails to measure the absolute amount absorbed. It permits the comparison of responses of the same individual to different iron preparations, to the same salt at different periods of hematopoietic activity, and under different gastro-intestinal influences. In health, under iron therapy an increase is apparent within the first hour after ingestion of relatively large doses, reaches maximum in two and one-half to five hours and then slowly disappears to reach basal values in twelve hours. It leaves no permanent increase in serum iron in health even after one or two months of therapy. Serum iron in pernicious anemia is a high normal or higher than normal and decreases to basal levels with liver therapy. A reported pernicious anemia serum iron of 5.8 mg. and a reported hypochromic anemia serum iron of 0.04 mg. per cent are a marked contrast to a normal serum iron of 0.113 mg. per cent.

Thus it would follow that serum iron values may be of use in differential diagnoses in the various anemias, and of importance in permitting one to interpret adequacy of iron reserves, rate of erythrogenesis, and rate of erythrocytic destruction.

B. The non-hemoglobin corpuscular iron (N.H.C.Fe) is called "leicht abspaltbaren Bluteisens"—"easily split-off blood iron" because it is thought to be split off easily from a decomposition product of hemoglobin and is destined for bile pigment formation. It may be included in the cell during its production in the bone marrow, or may be evolved during the breakdown of hemoglobin in the functional life of the red blood corpuscle. The real importance of this fraction of blood iron has been proven definitely *not* to be iron in transportation. Physiologically under basal conditions there may be as much as 15 per cent variation in one individual—"more of an individual than a pathological characteristic—not being effected by most procedures which ordinarily influence other iron fractions."[6]

It has been shown that non-hemoglobin corpuscular iron does

not vary with the hemoglobin level from one individual to another, nor does it parallel hourly changes in the hemoglobin concentration in the same individual. In iron therapy N.H.C.Fe does not contribute to change in serum concentration.—Moore.

Preliminary work has shown that in anemia, the non-hemoglobin corpuscular iron is higher than normal in all severe cases.[5]

TABLE IV
IRON CHEMISTRY DATA

| | Serum Iron | |
|---|---|---|
| | Normally | In This Study |
| Range in mg. per cent. | 1. 0.056-0.168, avg. 0.113 —Barkan<br>2. 0.07-0.18, avg. 0.125 —Moore<br>3. 0.04-0.23, avg. 0.13 —Walker<br>4. Females 0.8, males 0.3 —Jenkins-Thomson<br>5. Average — 0.126 —Heilmeyer-Plötner | 0.038-0.166<br>Avg. — 0.104 |
| Physiological variations in an individual daily and hourly under basal conditions. | Slight: 0.01-0.065 mg. per cent "of oscillatory nature and devoid of any direction diagnostically." | This phase excluded from this study |

| | Non-hemoglobin Corpuscular Iron (N.H.C. Fe) | |
|---|---|---|
| | Normally | In This Study |
| Range in mg. per cent | 2.4-10 per cent of total whole blood iron; on the basis of female Hb iron as 45.8 mg. % calculated range: 1.09-4.58 —Moore | 1.58-5.30<br>Avg. — 2.48 |
| Menstrual variations | Highest before and during; lowest immediately after | The stage in the menstrual cycle was taken, but since the readings were singly made per individual, there is no room for comparison. Furthermore, No. 49 menstruation intermittent: 2.17 mg. per cent<br>No. 50 no menstruation for the entire year: 2.16 mg. per cent |

### *Determination of Iron*[6]

Breuer and Militzer, Kennedy, Josephs, Jenkins and Thomson, and Barkan and Walker have given improved methods for determining iron in blood. All are colorimetric in nature, Barkan and Walker adapting their procedure to the photelometer. In this study an adaptation of Barkan and Walker's method is made and this adaptation employed to avoid objections sometimes raised against the methods using thiocyanate, or methods using oxidants in wet or dry treatment, or using alternately different mineral acids, or the ones employing thioglycollic acid.

**Serum Iron**
**Barkan and Walker (An adaptation)**

Reagents:

1. One and two-tenths per cent hydrochloric acid solution (aq.)
2. Twenty per cent trichloracetic acid solution (aq.)
3. Saturated solution of sodium acetate
4. 2 M acetate buffer (pH 4.5)
   2 M sodium acetate solution 90 volumes
   2 M acetic acid solution 110 volumes
5. One per cent hydrazine sulphate in 2 M acetate buffer sol. (Prepare daily)
6. One-tenth per cent ortho-phenanthroline monohydrate sol.* (Warm to hasten solution. Reject if colored. Keeps several weeks on ice.)
7. Stock iron solution
   Iron wire C.P. 50 mg.
   Dist. water q.s.ad. 100 cc.
   Conc. 1 cc. = 0.5 mg.

Standard iron solution No. 1
Stock iron solution 10 cc.
Dist. water q.s.ad. 1000 cc.
Conc. 1 cc. = 0.005 mg.

Set-up:

Serum or plasma 2 cc.
1.2 Per cent HCl sol. 1 cc.
Incubate, stoppered overnight at 37° C.
Cool to room temperature and add
20 Per cent trichloracetic acid 1 cc.
Let stand at room temperature 1 hr.
Cover with tinfoil stopper and centrifuge 15 min. at 3000 R.P.M.

Standard iron solution No. 2
Standard iron sol. No. 1 0.1 cc.
Dist. water 1.9 cc.
Use in TEST as indicated immediately below

---

* Ortho-phenanthroline monohydrate may be purchased from G. Frederick Smith Chemical Company, 867 McKinley Ave., Columbus, Ohio. P.O. Box 1611. $2.25/gm.

| Test: Unknown | | Standard | |
|---|---|---|---|
| Clear supernatant fluid | 2 cc. | Standard iron sol. No. 2 | 2.0 cc. |

To both add

| | | |
|---|---|---|
| Sat. sodium acetate sol. | 0.5 cc. | Mix well |
| Hydrazine sulphate sol. | 0.5 cc. | |
| O-phenanthroline monohydrate | 0.5 cc. | |

Let stand stoppered at room temperature 1 hr. Read colorimetrically.

Calculation: $\frac{S}{R} \times 100 \times 0.0005$ mg. = mg./100 cc.

Non-hemoglobin Corpuscular Iron

Set-up:

| | | |
|---|---|---|
| Whole blood | 2 cc. | Incubate overnight at 37° C. |
| Dist. water | 8 cc. | |
| 1.2 Per cent HCl | 5 cc. | |

Cool to room temperature and add

| | | |
|---|---|---|
| 20 Per Cent trichloractic acid | 5 cc. | Thoroughly mix and let stand rm. temp. 1¼ hr. |

Filter through ashless filter

| Test: Unknown | | Standard | |
|---|---|---|---|
| Filtrate | 10 cc. | Standard iron sol. No. 1 | 0.5 cc. |
| | | Dist. water | 9.5 cc. |

To both add

| | | |
|---|---|---|
| Sat. sodium acetate sol. | 1 cc. | Mix well and let stand at rm. temp. 1 hr. |
| Hydrazine sulphate sol. | 1 cc. | |
| O-phenanthroline sol. | 1 cc. | |

Read colorimetrically.

Calculation: $\frac{S}{R} \times \frac{300}{4} \times 0.025$ mg. = mg./100 cc.

Discussion:

The chemistry of Barkan and Walker's method is essentially, in order of step sequence:

1. An action on the labile iron by means of 1 hour (preferably overnight) incubation with weak HCl solution at 37° C.

2. Precipitation of any proteins by trichloracetic acid, then

3. Changing the pH by addition of saturated sodium acetate solution to favor

4. Subsequent reduction of the iron by hydrazine sulphate in reduced acidity without which, reduction cannot be accomplished, and

5. Tying up of ferrous iron with o-phenanthroline monhydrate to yield a pink complex, easily readable colorimetrically with the use of a cobalt filter between the eye and eyepiece.

Remarks:

1. The reagents must all be free from iron contaminations. Chemically pure reagents can so be procured with the exception of trichloracetic acid, and the contamination due to it rules itself out in the colorimetric adaptation.

2. The 1.2 per cent HCl in the set-up makes the plasma approximately equal to 0.1 N HCl, volume for volume, which in 15-30 minutes and preferably overnight incubation ionizes the iron or so changes it that it passes quantitatively into the filtrate when trichloracetic acid is added as protein precipitate.

3. Oxalates are not suitable for anticoagulants.

4. The protein precipitation is never complete in less than 1¼ hours.

5. Traces of hemoglobin in serum or plasma are no source of error as hemoglobin is precipitated as protein by the trichloracetic acid.

6. Plasma or serum must be separated from cells within an hour.

7. The color development is complete in an hour and stable for 8 days.

Conclusion:

In the limited amount of experimentation done with serum iron and non-hemoglobin corpuscular iron in this study (49 determinations of each) the only conclusions that can be drawn are confirmatory of the findings of research workers in the field.

1. That normally the serum iron values average about 0.110 mg. per cent;

2. Non-hemoglobin corpuscular iron values are considerably higher than serum iron.

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Adaptation of technique from Barkan, G. and Walker, B. S.: "The Determination of Serum Iron and Pseudohemoglobin Iron with O-phenanthroline," *J. B. C.* XXXVII (1940), 41.

CHART II

(Scattergram)

Correlation of Hemoglobin and Total Acidity Data

| | 10-20 | 20-30 | 30-40 | 40-50 | 50-60 | 60-70 | 70-80 | $F_x$ | $D_x$ | $FD_x$ | $FD_x^2$ | $\Sigma x'y'$ + | $\Sigma x'y'$ − |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13.5 – 14.0 | | 1 | 1 | 1 | | | | 3 | 2 | 6 | 12 | 2 | -2 |
| 13.0 – 13.5 | 1 | 3 | 10 | 8 | | | 1 | 23 | 1 | 23 | 23 | 12 | -5 |
| 12.5 – 13.0 | 2 | 4 | 14 | 12 | 4 | | | 36 | 0 | 0 | 0 | 0 | 0 |
| 12.0 – 12.5 | | 9 | 5 | 3 | 1 | 2 | | 20 | -1 | -20 | 20 | 9 | -11 |
| 11.5 – 12.0 | 2 | 2 | 3 | 5 | 1 | | 1 | 14 | -2 | -28 | 56 | 12 | -22 |
| 11.0 – 11.5 | | 1 | 1 | | 1 | | | 3 | -3 | -9 | 27 | 3 | -6 |
| 10.5 – 11.0 | | | 1 | | | | | 1 | -4 | -4 | 16 | | |
| $F_y$ | 5 | 20 | 35 | 29 | 7 | 2 | 2 | N=100 | | | 154 | | |
| $D_y$ | -2 | -1 | 0 | 1 | 2 | 3 | 4 | | | | | | |
| $FD_y$ | -10 | -20 | 0 | 29 | 14 | 6 | 8 | 27 | | | | | |
| $FD_y^2$ | 20 | 20 | 0 | 29 | 28 | 18 | 32 | 147 | | | | | |

*Comparison and Conclusion*

Since hypochlorhydria and achlorhydria are characteristically associated with hypochromic anemia, the data of this study was mathematically treated to show such a correlation or lack of it. Chart II gives the synopsis of the mathematics involved. The central figure is the number of cases which lie between the limit of hemoglobin (in grams) on the left and the degrees of total acidity at the heads of the columns. The guessed averages are those numbers lying in the paths bordered by heavy vertical and horizontal lines. The variations calculated on the basis of the guessed averages are represented by the digits in the upper right hand corners of every square; the product moment (number of cases $\times$ variations) is in the lower left hand corner of each square.

The product moments were algebraically summed. The sums were small and negative, whereas, had there been positive correlation between acidity and hemoglobin, these summations would probably be large and positive. By mathematically treating these

sums with other statistical data, employing a correction for the guessed averages, a coefficient of correlation was calculated.

A perfect positive correlation would give a coefficient of 1.00. The coefficient of correlation obtained from treatment of statistics of this study was 0.0045, which means there is absolutely no relationship shown.

The volunteers used in this study may not have represented an unselected group (an unselected group is a necessity for correlating one abnormal function with another abnormal function. Many of these came to participate because either they themselves or their nearest kin (father, mother, sister, brother) were suffering or had in the past suffered from gastro-intestinal disorders or poor blood conditions.

This group might represent the lower corner of a distribution, for the entire number of scores just attain or lie below a hemoglobin level of 13.5-14.0 gm. It is possible with a larger and more unselected group that additional cases would lie in the upper right hand corner of the scattergram, thus giving a positive trend in the correlation. If this should be true, it might bring about a coefficient of correlation approaching 1. Thus, in the cases presented in this study, it has been shown that there is no correlation.

It has been unofficially revealed that in a five-year project now closing in which the nutritional status of high school and college girls is being studied cooperatively by experimental stations of institutions of higher learning in the following states: Kansas, Iowa, Ohio, Nebraska, Oklahoma, and Minnesota, the high school students from the various states showed a lower mean count and hemoglobin than college girls; that students from the various states represented similar dietary patterns; that week-end diet deviations were prevalent; and that in general the diets were low in milk and eggs.

While the present study gives the reverse picture of high school girls, there is a wholesome anticipation that perhaps when the complete report of this five-year study is published young women throughout this nation may present a similar hematological pattern and that instead of ruling out lack of acid in the stomach, emphasis will be placed on adequate diet and rest, with reduced iron thera-

peutically and even prophylactically, to avoid hypochromic anemia in any emergency.

*Summary*

It has been shown in this study that in females of ages 15-23 years inclusively

1. The incidence of achlorhydria is no greater than what constitutes the normal condition in other studies.
2. That not all stomachs have identical rates of elaboration of secretions.
3. That the hematological status is low normal when compared with statistics available.
4. That no correlation could be found between the status of the blood stream and the acidity of the gastro-intestinal tract.

Grateful acknowledgment is made to Dr. E. L. Tuohy for proposing this field for research, for timely sources of inspiration, and for criticizing very constructively the manuscript; to the Sisters of the College and the Hospital for their splendid assistance in the many details this study involved; to the students who participated; to the junior interns in medical technology who rendered much technical assistance; to Miss Marie Fraser for mathematical treatment of statistical data; and lastly to Miss Anne Biglow and Miss Gertrude Liebl for their splendid clerical help.

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*Jour. Biol. Chem.*, LXXIV (1927), 385.

# CHEMICAL INACTIVATION OF AUTOGENOUS VACCINES

By J. N. FRAZER, M.T.

*From the Laboratory of the Union County Hospital, Darrel L. Vaughn, M.D., Director, Morganfield, Ky.*

The use of chemicals in inactivating autogenous vaccines has not received widespread attention. This laboratory has made a number of these vaccines, all of which have given excellent results. Nothing original is presented here, but a unique detailed method is outlined. Also, mention of the results of workers with chemically inactivated autogenous vaccines is made.

There are several advantages in using chemically inactivated vaccines. It is possible to produce a vaccine containing a suspension of completely inactivated bacteria that are nearly identical, antigenically and otherwise, to the living organisms. Such vaccines are much more potent and effective than vaccines killed with heat. Viruses can be easily incorporated into these vaccines.

Powell and Jamieson have done considerable work, in the laboratories of Eli Lilly and Company, on the use of chemicals in inactivating suspensions of bacteria. A recent publication[1] gives a review of much of their work. Merthiolate (Sodium Ethyl Mercuri Thiosalicylate, Lilly) was used in their experiments.

These workers, in an earlier paper[2], have shown that vaccines prepared with Merthiolate still have the very labile antigen fraction intact. In this publication, they showed such vaccines to have a higher titer than those killed by heat.

Merthiolate has been successfully used as a preservative of bacteriophages (3), toxins (2), toxoids (4), serums (2), and vaccines (2). Rosenstein and her associates (1) have used Merthiolate 1-20,000 plus 0.25% phenol, and observed that the two chemicals seem to fortify each other.

Our laboratory has prepared a number of vaccines using Mer-

thiolate plus phenol. The method used is as follows:

(1) The organism in pure culture (several species of organisms in pure culture may be treated similarly) is spread over six slants of appropriate culture media.

(2) After 24 hours incubation, if the growths of bacteria are moderate or profuse, a few cubic centimeters of sterile saline is added to each tube and the growths are scraped off with sterile applicators.

(3) The resulting suspension is filtered through sterile cotton (an ordinary centrifuge tube, with the bottom cut off, serves as an efficient holder for a small wad of cotton).

(4) The suspension is filtered at high speed until the supernatant fluid is clear and the organisms are packed into the end of a sterile graduated centrifuge tube.

(5) The supernatant fluid in the tube is poured off, and sufficient sterile saline added to make a 1% emulsion of the organisms.

(6) The vaccine is made,

| | |
|---|---|
| 1% emulsion of organisms | 2 cc. |
| 1% phenol in 0.85% NaCl | 5 cc. |
| Sterile saline | 12 cc. |
| Merthiolate Solution 1-1000 | 1 cc. |

(For the more pathogenic organisms, it may be necessary to use 1 cc. of the emulsion of organisms, rather than 2 cc. This is usual when pneumococci, acne, or pyocyaneous are put into a vaccine.)

(7) This finished vaccine is set at room temperature for 24 hours. Then, it is cultured for sterility on slants of appropriate culture media. These slants are heavily inoculated with the vaccine, in order to overcome the inhibitory action of the chemicals (1).

Note: Powell and Jamieson incubated their sterility tests for seven days (2). Some may prefer to shorten this time.

Caution should be observed when using any other mercurial chemical, as some will precipitate if brought beyond their usual

alkaline range. Merthiolate is still active used as above, but it will precipitate if put in strongly acid solutions.

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# ABSTRACTS

**THE ABSORPTION AND EXCRETION OF SULFAPYRIDINE AND OF SODIUM SULFAPYRIDINE IN MAN: H. D. Ratish, A. Davidson and J. G. Bullowa, Jr. Pharm. & Exp. Therapeutics, vol. 69, No. 4, Aug., '40, p. 365.**

Sodium sulfapyridine produced the desired blood levels in less than 2½ hours, whereas sulfapyridine required 12-24 hours. It also produced less acetyl sulfapyridine in the blood than did sulfapyridine. No gastro-intestinal irritation other than nausea and vomiting were found.

**THE CHEMOTHERAPY OF EXPERIMENTAL HEMOLYTIC STREPTOCOCCAL INFECTIONS WITH GOLD SALTS: M. H. Dawson and G. L. Hobby, Jr. Pharm. & Exp. Ther., vol. 69, No. 4, Aug., '40, p. 359.**

Aurosodium thiomalate showed marked chemotherapeutic properties against hemolytic streptococcal infections in mice. Tolerance was 10X the therapeutic dosage. In vitro its bacteriostatic effect was comparable with that of sulfanilamide and sulfapyridine. No bactericidal effect was demonstrable.

**THE INHIBITION OF THE BACTERIOSTATIC ACTION OF SULFONAMIDE DRUGS BY SUBSTANCES OF ANIMAL AND BACTERIAL ORIGIN: C. M. MacLeod, Jr. Exp. Med., vol. 72, No. 3, Sept., '40, p. 217.**

Extracts of fresh muscle, pancreas and spleen contained inhibitor while liver was found to be free from it. Autolysis of tissue increased the amount of inhibitor. Acid hydrolysis developed it in human urine which is normally free. In some bacteria inhibitor was found to occur in the cells but not in the culture medium while in others the reverse was true. Increased sulfonamide inhibitor was demonstrated with the development of sulfapyridine fastness in a Pneumococcus Type I strain.

**INFECTIOUS MONONUCLEOSIS: R. R. Kracke, Texas State Jr. Med., vol. 36, No. 5, Sept., '40, p. 348.**

Discussion of reported facts. *Incidence:* age 6-70 with most in young adults. Males and females equally affected. Occurs mostly in white people, rarely among negroes. Reported from practically all over the world with no seasonal incidence.

Etiology: cause still unknown.

Clinical manifestation: vary greatly but the absence of any lymphadenopathy is not likely. Recently central nervous system symptoms have been reported, some even showing spinal fluid with several hundred lymphocytic cells per cmm.

Blood findings: WBC 10,000-20,000, rarely up to 60,000. Absolute and relative lymphocytosis with the characteristic cell large, irregularly lobulated and having sky blue cytoplasm with a perinuclear clear zone. They may also have small vacuoles in the cytoplasm. These cells may make up 50% or more of the lymphocytes.

Heterophile antibody titer should be 1:32 or more.

Falsely positive Wassermann reactions occur in 15-20%. Antibodies against B. typhosus, B. paratyphosus, B. suipestifer, etc., may also occur.

**SURGICAL MANIFESTATIONS OF AMEBIASIS: R. W. Mendelson, Southwestern Medicine, vol. 23, No. 11, Nov., '39, p. 361.**

Liver abscess is the most frequent, intestinal obstruction next, due either to a stricture or to formation of a tumor. Intestinal perforation may also occur giving rise to peritonitis or fecal fistula. Pulmonary abscess may also occur usually as an extension through the diaphragm of a liver abscess.

# BOOK REVIEWS

**APPROVED LABORATORY TECHNIC, Clinical Pathological, Bacteriological, Mycological, Parasitological, Serological, Biochemical and Histological. By John A. Kolmer, M.S., M.D., Dr.P.H., Sc.D., LL.D., L.H.D., F.A.C.P., Professor of Medicine, Temple University; Director of the Research Institute of Cutaneous Medicine, Philadelphia; Formerly Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania and Fred Boerner, V.M.D., Assistant Professor of Bacteriology, School of Medicine and Graduate School of Medicine, University of Pennsylvania; Bacteriologist, Graduate Hospital, Philadelphia, with Twenty Eight Collaborators. Third Edition, 1941. Pp. 921. D. Appleton-Century Company, Inc., 35 West 32nd Street, New York, N. Y. Price $8.00.**

This third edition since 1931 attests to the popularity of this work. All technics described have been approved by at least five members of The American Society of Clinical Pathologists, hence the title "Approved" Laboratory Technic. Throughout the work the authors must be commended for their meticulous attention to detail of description and at the same time simplicity of presentation with stress placed on the use of accurate and reliable apparatus and reagents. They also point out possible sources of error and how to avoid them. In the present edition many minor changes have been made throughout the text. A twenty-seven page appendix has been added to include the new and approved methods. These are MacKenzie's modicafition of the Donath-Landsteiner test for paroxysmal hemoglobinuria; Quick's method for quantitative determination of prothrombin; methods for the examination of semen; methods for the determination of urobilinogen in the feces and urine and of hippuric acid in urine for liver function; determination of serum lipase for pancreatic disease; simplified Kolmer complement fixation test; Eagle modification of the Wassermann test; determination of vitamin C in urine and plasma; methods for the determination of the various sulfonamide preparations in the blood and urine; method

for the determination of thiocyanates in the blood. Because this work is authoritative and because all phases of laboratory technic are so thoroughly covered it is useful to medical students, physicians, teachers, clinical pathologists and laboratory technicians.

**HANDBOOK OF LABORATORY TECHNIC. By Josephine M. Galloway. 258 Pages. 46 Illustrations. Bibliography. F. A. Davis Company, Philadelphia, 1941. Price $3.00.**

This handbook has been prepared with a knowledge of the adjustment problems of the inexperienced laboratory technician from the theoretical to the practical; of the requirement that technicians possess correlative knowledge as well as technical skill. It is arranged for quick, convenient use, both for confirmatory tests and as a correlative reference for interpretation.

Part I covers the general laboratory tests with which every technician should be familiar including such subjects as Hematology, Urinalysis, Sputum, Cerebrospinal Fluid, Pathogenic Bacteria, etc.

Part II contains an alphabetical listing of diseases with their respective laboratory findings.

Part III covers the latest laboratory procedures, particularly involved in the use of the sulfanilamides and the new discoveries concerning the vitamins.

**THE PHARMACOLOGY OF ANESTHETIC DRUGS. A Syllabus for Students and Clinicians by John Adriani, M.D., Instructor in Anesthesia, New York University College of Medicine, Assistant Visiting Anesthetist Bellevue Hospital. Second Edition, 1941. Pp. 86. Charles C. Thomas, Publisher, Springfield, Ill. Price $3.50 postpaid.**

Presented in syllabus form with anatomical and other diagrams this book gives in outline the pharmacological action of all the known anesthetic and analgesic drugs and of the non-anesthetic drugs used in conjunction with anesthesia. It is the object of the author to acquaint the student anesthetist with pharmacological facts pertaining to the use of these drugs and to stress the physiological and pathological changes that occur in various organs and systems. By presenting the subject in an atlas manner with diagrams the author

admirably accomplishes his purpose. The pertinent facts can be found at a glance with lines drawn to the particular organ or tissue concerned on the anatomical diagram. All statements are brief and to the point and are accurate so far as our present knowledge permits. Controversial points are so stated. The various theories of narcosis are briefly presented at the outset of the book. The action of the barbiturates in general is given and those commonly used are individually treated. With the continuation of the old and the wide acceptance and use of many new anesthetic agents this work makes a timely encyclopedia of very practical information. With the advent of these new drugs the anesthetic used can now be better fitted to the condition of the patient and to the operation to be done. It behooves anesthetist and surgeon alike to be well versed in the pharmacological action of these agents and to know the indications and contra-indications for their use. In this respect the book fills a definite and timely need.

# NEWS AND ANNOUNCEMENTS

## NOTICE—A. S. M. T. MEMBERS

Order blank and fac-simile of *Automobile Emblem* adopted by the organization for exclusive use of members of the American Society of Medical Technologists may be found in front advertising section of this issue.

## NEW CLAY-ADAMS CENTRIFUGE CATALOG NOW AVAILABLE

The Clay-Adams Co., Inc., New York, have just issued their new 16-page Catalog No. 111—printed in 2 colors, featuring their entire Centrifuge line. If you have not received your copy, you may obtain one by writing to the company on your department letterhead.

### *Ohio*

The Ohio Society of Medical Technologists have voted upon the publication of a quarterly bulletin to be released soon. In addition to the meeting held in April with the Ohio Hospital Association, a fall meeting will be held in October. The president for the year is Carolyn Hilles, University Hospital, Columbus, O. The editor of the bulletin is Mrs. Robt. Harsh, also of University Hospital.